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(71) Applicant *(for all designated States except US)*: MEDEA RESEARCH S.R.L. [IT/IT]; Via Cappuccini, 20, I-20122 Milano (IT).

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(54) Title: ANTIARTERIOSCLEROTIC AGENT, A PROCESS FOR THE PREPARATION THEREOF AND THE USE THEREOF

(57) Abstract

3-Acetoxythiophene-2-carboxylic acid, a process for the preparation thereof and the use thereof in human therapy.

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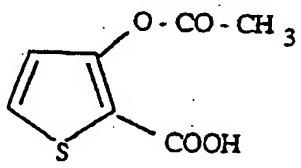
ANTIARTERIOSCLEROTIC AGENT, A PROCESS FOR THE PREPARATION THEREOF AND THE USE THEREOF

The present invention relates to 3-acetoxy-thiophene-2-carboxylic acid, which will hereinafter be named MR-2058, of formula



(I)

and to the pharmaceutically acceptable salts thereof. The invention also relates to a process for the preparation of compound MR-2058, to pharmaceutical compositions containing them and to the use thereof in the preparation of medicaments useful in the treatment of arteriosclerosis.

The compound of the invention is characterized by the presence of a carboxy group; therefore the present invention also relates to all the possible salts of the acid with non toxic, pharmaceutically acceptable organic and inorganic bases. Examples of said salts are the sodium, potassium, calcium, iron, zinc salts; as well as those with diethylethanolamine, morpholine, piperidine, triethylamine.

The migration and proliferation of the smooth muscle cells (SMC) of the arterial wall are the basic events in pathogenesis of the main cardiovascular diseases, such as hypertension, atherosclerosis and those accelerated atherosclerosis syndromes often occurring after coronary by-pass and angioplasty.

Therefore, substances which can interfere in said processes are highly requested.

Now, it has been found that MR-2058 has a surprising activity inhibiting proliferation of SMC of the arterial wall, and it also has other interesting physiological properties.

Antiarteriosclerotic effects

MR-2058 proved to have a marked ability to inhibit the proliferation of SMC from rat aorta, in the test carried out according to the procedure described by Bernini et al. (Pharm. Res. 1, 27-35, 1990).

Anti-platelet aggregation activity

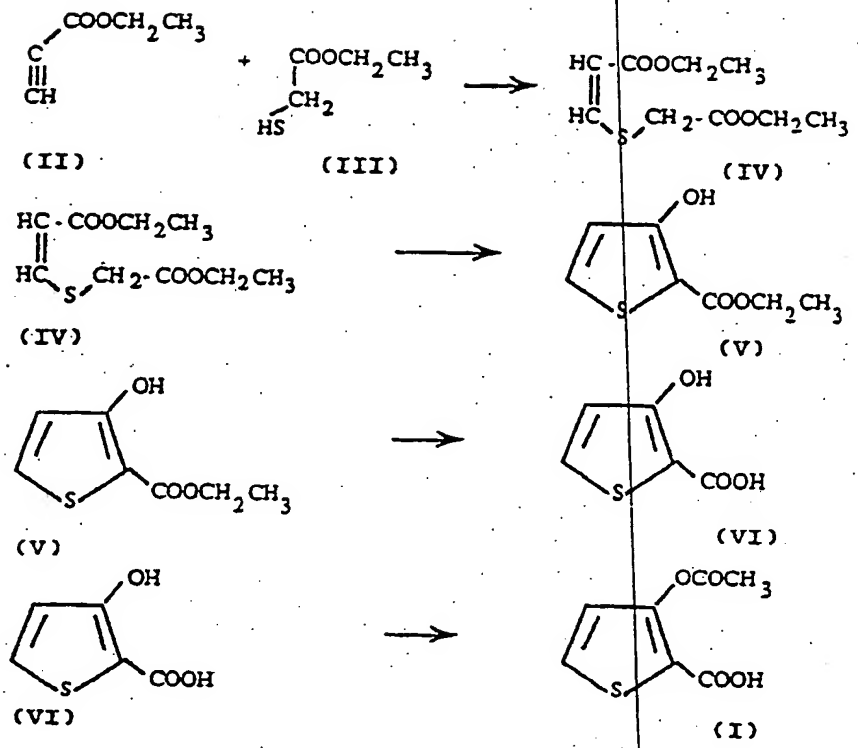
In the tests according to Garbin et al. (Pharmacol. Res. Comm. Vol. 15, No. 1, 1983), MR-2058 was found to have an anti-platelet aggregation activity comparable to that of acetylsalicylic acid.

Antithrombotic activity

The experimental model described by Kohler and coll. (Tromb. Res. 9, 67-80; 1976) was used, consisting in evaluating the percent protection against death induced by arachidonic acid in the rat.

In the group of animals treated with MR-2058, a high percentage of thrombosis inhibition was evidenced, which percentage being greatly significant compared to the controls, and higher than that of the control drugs.

The process for the preparation of MR-2058, according to the invention, is shown in the scheme below.



(II) (III) (IV)

(IV) (V)

(V) (VI)

(VI) (I)

Ethyl propiolate (II) is condensed with ethyl thioglycolate (III) in an equimolar ratio. The resulting compound (IV) is cyclized to ethyl 3-hydroxythiophene-2-carboxylate (V); the subsequent hydrolysis of the ester and the acetylation of the hydroxy group at the 3-position give MR-2058 (I).

The reaction of (II) and (III) is carried out in a medium consisting of an aqueous-organic homogeneous phase, comprising an organic solvent mixed with water in various ratios; solvents such as methanol, ethanol, acetone, dioxane can be used, a 1/1 (v/v) ethanol-water mixture being preferred. This reaction is promoted by the presence of an acid-binding agent, such as

trimethylamine, triethylamine, pyridine. Compound (III) is present in the two isomeric cis-trans forms; however the isomeric mixture is directly used in the subsequent step.

Cyclization of (IV) occurs via the carbanion, and it is carried out with conventional methods in which such an intermediate is formed, i.e. in the presence of a strong base, such as an alkali alkoxide, for example sodium methoxide, in anhydrous solvents, such as benzene, toluene, xylene.

The preparation of (V) is an alternative method to the one described in literature (Berichte 87, 841; 1954), which consists in preparing (V) directly from ethyl propiolate and thioglycolate in benzene, with sodium ethoxide, with a 30% yield. On the contrary, the isolation of (IV) and its subsequent cyclization gave (V) in a 67% total yield.

Hydrolysis of (V) to (VI) is carried out at a temperature from 50 to 60°C; a higher temperature causes pitch to form, whereas at lower temperatures the reaction does not proceed. In literature (Berichte 87, 841; 1954) the hydrolysis carried out at 100°C gives (VI) in a 35% yield. Product (VI) is unstable and it undergoes darkening and decomposition with time, even if it is shielded from air and light. Therefore, it must be reacted as soon as prepared.

The following example further illustrates the invention.

Example

3-Acetoxythiophene-2-carboxylic acid

1) Ethyl 3-(2-ethoxycarbonylethyl)thio-2-propenoate

To a mixture of 11.2 ml (0.1 mole) of ethyl thioglycolate, 100 ml of $EtOH/H_2O$ (1/1 - v/v) and 5 drops of triethylamine, 10.4 ml (0.1 mole) of ethyl propiolate are added dropwise. The mixture is left under stirring at room temperature for 3 hours. Solvent is evaporated off, stripping water, if any, with toluene. (IV) Is obtained as a slightly yellow liquid.

NMR analysis evidences the presence of the two cis/trans isomers in a 77/23 ratio. The product is directly used for the subsequent step.

Yield: 22 g (quantitative)

NMR ($CDCl_3$): in agreement

TLC (Ph CH_3/AcOEt = 9/1): unitary Rf = 0.3

2) Ethyl 3-hydroxythiophene-2-carboxylate

7 g (0.13 mole) of sodium methoxide are suspended in 80 ml of anhydrous toluene. 22 g (0.1 mole) of (IV) dissolved in 10 ml of anhydrous toluene are dropped therein, under strong stirring.

The reaction mixture is left to react for 1 hour, after which a red solution is obtained, which is ice-cooled and adjusted to pH 2 with conc. hydrochloric acid. The mixture is stirred for 10 min., then the two phases are separated. The aqueous phase is washed with 20 ml of toluene. Solvent is evaporated off the organic phase, to yield 21.3 g of a dark orange liquid which is distilled under reduced pressure, recovering the fraction boiling at a temperature of 115-120°C (p = 20 mm Hg). (VI) Is obtained in the form of a light yellow liquid.

Yield: 11.54 g (67%) (Lit. bp = 109°C/16 mm Hg)

NMR ($CDCl_3$): in agreement

TLC (AcOEt - 9/1): unitary Rf - 0.6

3) 3-Hydroxythiophene-2-carboxylic acid

11.54 g (0.0671 mole) of (V) are dropped into 85 ml of 4N aqueous NaOH. When the addition is over, the reaction mixture is heated on bath at 60°C for 7 hours (brown solution). The mixture is ice-cooled and acidified to pH 2 with conc. HCl: a solid precipitates which is filtered and dried in the air, to obtain (VI) as a light pink solid.

Yield: 5 g (52%)

M.p.: 112-114°C dec. (lit. 108°C)

TLC (toluene/dioxane/AcOH - 45/10/2): unitary Rf - 0.25

NMR ($CDCl_3$ + DMSO) and I.R. (nujol): in agreement

4) 3-Acetoxythiophene-2-carboxylic acid

A mixture of 4.62 g (0.0321 mole) of (VI) and 7 ml of acetic anhydride is kept under stirring at room temperature for 6 hours. The resulting brown solution is washed with water and extracted with ethyl ether. The organic phase is dried and evaporated. The resulting residue is chromatographed on 150 g of silica gel using n-hexane/ethyl ether - 1/1 as eluent. The obtained pink solid is crystallized from diisopropyl ether. (I) Is obtained as a nearly colorless solid.

Yield: 3.3 g (55%;

TLC (n-hexane/ethyl ether - 1/1): Rf - 0.1 unitary (toluene/dioxane/AcOH - 45/10/2): Rf - 0.3, slight impurity at a higher Rf

I.R.(nujol): 1650 cm^{-1} (ν c - 0 -COOH) 1760 cm^{-1} (ν c - 0 -O-CO-CH_3)

Elementary analysis for $C_7H_6O_4S$

% calc. C 45.16 H 3.23

% found C 45.23 H 3.28

NMR ($CDCl_3$) 2.3 δ s 3H ($-OCOCH_3$), 6.9 δ d 1H (CH = CH-S), 7.6 δ d 1H (CH - CH-S), 7.7 δ broad sign. 1H (-COOH).

Note

Product (4) and its precursor (3) have substantially the same Rf with many eluents. They differ in the iodine adsorption ((3) adsorption being higher). Therefore, in order to follow the reaction progress (step 4) by TLC, even though the products do adsorb UV light, it is necessary to use iodine as the developer after the plate has been eluted twice in n-hexane-ethyl ether = 1/1.

Salification of the carboxy group of (I) can be carried out with conventional techniques. The corresponding sodium, potassium, ethanolamine salts were prepared.

The present invention also relates to pharmaceutical compositions containing compound MR-2058 as the active ingredient, alone or in admixture with conventional carriers and excipients, according to the techniques described, for example, in "Remington's Pharmaceutical Sciences Handbook" Mack. Pub. Co., N.Y. U.S.A.

Examples of pharmaceutical compositions are soft and hard gelatin capsules, tablets, optionally in gastro-resistant or slow-release forms, powders, solutions and suspensions for the oral and parenteral administrations, suppositories, sustained-release forms.

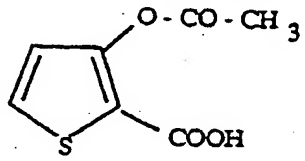
The pharmaceutical carriers can be excipients for solid forms, such as lactose, talc, PVP; granulating agents, such as magnesium stearate; suspending agents, such as methyl cellulose; and/or surfactants, such as polyoxyethylene stearate; preservatives, such as hydroxybenzoates; flavoring and sweetening agents.

The compositions of the invention are formulated preferably in unitary dosage forms, containing a therapeutically effective amount of MR-2058.

The daily dosage will depend on the severity of the disease to treat, as well as on the patient's conditions.

CLAIMS

1. 3-Acetoxythiophene-2-carboxylic acid of formula



(I)

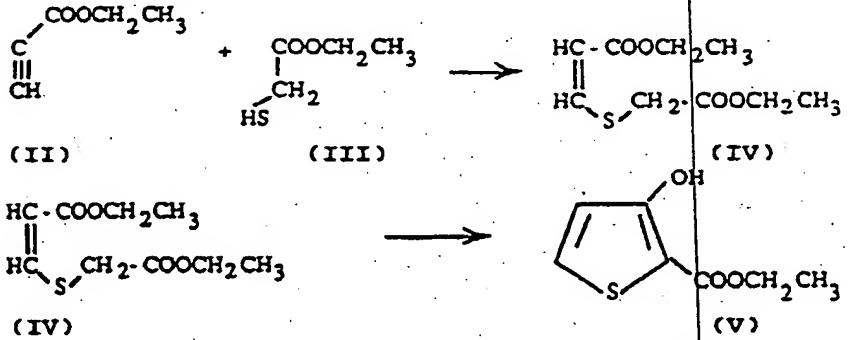
and the pharmaceutically acceptable salts thereof.

2. A process for the preparation of the compound of claim 1 characterized in that:

a) ethyl propiolate (II) is condensed with ethyl thioglycolate (III);

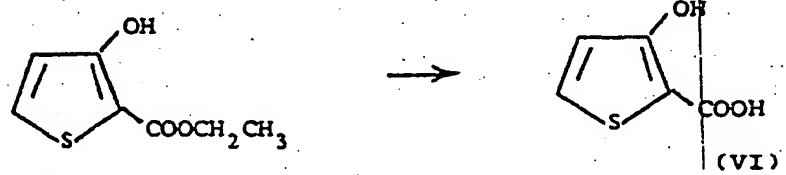
b) the resulting intermediate (IV) is cyclized to ethyl 3-hydroxythiophene-2-carboxylate (V);

c) (V) is subsequently hydrolyzed and acetylated at the 3-hydroxy group, according to the following scheme:

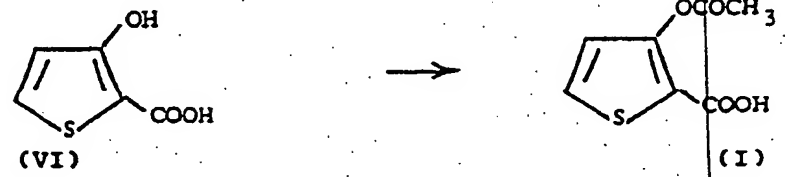


(II) (III) (IV)

(IV) (V)



(V) (VI)



(VI) (I)

3. A process according to claim 2 characterized in that ethyl propiolate and ethyl thioglycolate are reacted in a 1/1 (v/v) ethanol-water mixture in the presence of triethylamine and the obtained compound is recovered from the reaction mixture, then it is cyclized to ethyl 3-hydroxythiophene-2-carboxylate via carbanion using sodium methoxide in anhydrous toluene.

4. Pharmaceutical compositions containing the compounds of claim 1 as the active ingredient, optionally in admixture with pharmaceutically acceptable carriers and excipients.

5. The use of the compounds of claim 1 as therapeutical agents.

6. The use of the compounds of claim 1 for the preparation of a medicament for the treatment of arteriosclerosis.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 92/00713

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07D333/38; A61K31/38

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C07D

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[10]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
A	DE,B,1 020 641 (BASF AKTIENGESELLSCHAFT) 12 December 1957 see the whole document ---	2
A	CHEMISCHE BERICHTE. vol. 87, 1954, WEINHEIM DE pages 841 - 848; H. FIESSELMANN ET AL.: 'Über Oxythiophen-carbonsäureester, II. Mitteil.: Synthese und Reaktionen von 3-Oxy-thiophen-carbonsäure-2-estern' cited in the application see the whole document --- -/--	1-3

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25 MAY 1992	04. 06. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	PAISDOR B.

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Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	J. MARCH 'Advanced Organic Chemistry, 3rd Edition' 1985 , JOHN WILEY & SONS, INC. , NEW YORK, US see chapter 10, pages 255 - 446; pages 347 - 348, paragraph 0-23 ---	1,2

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